2. Claims 22-24 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious in view of Coico *et al.* and Matsumura *et al.*

1. THE CLAIMS ARE ENABLED

Claims 1-8 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter not enabled by the specification. Specifically, the Examiner alleges on page 5, paragraph 11, that the "specification does not give any definitive evidence that measurement of α-dystroglycan is indicative of potential tumorigenicity" for several reasons. The Examiner therefore alleges that it would be unpredictable to measure potential tumorigenicity on α-dystroglycan levels because "There is no record in the art teaching dystroglycan as a tumor suppressor." In addition, the Examiner alleges that the specification "does not disclose whether a mutated form of the dystroglycan gene results in cancer" and "does not provide guidance as to what level of α-dystroglycan would constitute abnormal levels and how these levels would be indicative of potential tumorigenicity." (Office Action, page 5, paragraph 11).

Applicants are aware that the "role of dystroglycan as a tumor suppressor was, until now, entirely uninvestigated." (Specification, page 15, end of first paragraph). Prior art showing dystrogylcan's role in regulation of cell growth and cytoskeletal architecture in response to laminin in normal tissues is what led the inventors to study dystroglycan and its role in tumor cell growth. This newly found role of dystroglycan and dystroglycan-proteolysis in tumor cell growth is the reason for this application.

In response to Examiner's allegation that the "specification does not disclose whether a mutated form of the dystroglycan gene results in cancer, it only discloses many tumor cells do not have normal α-dystroglycan on the surface," Applicants assert that demonstration of a genetic mutation is not necessary to demonstrate enablement to a person skilled in the art. The Examiner has alleged that one of ordinary skill in the art would not be able to determine what would constitute a relative decrease of α-dystroglycan as compared to β-dystroglycan as

indicative of potential tumorigenicity, but appears to allege that in the absence of a specific number, the disclosure is not enabling. As the Examiner is of course aware, the burden is on the Office to establish a reasonable explanation as to why the scope of protection provided by a claim is not adequately enabled by the disclosure. Applicants respectfully assert that this burden has not been met. Detailed examples for using the invention are not necessary if the description of the invention is sufficient to permit those skilled in the art to use the invention. MPEP §2164.02. The test for determining enablement, as the Office correctly notes, is whether undue experimentation is needed to practice the invention. *In re Wands*, 858 F.2d 731, 737; 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

In this case, proteolytic cleavage causing α-dystroglycan to be shed from the cell surface is one event that causes disruption to dystroglycan function, and thereby results in a tumorigenic phenotype. See specifically pages 11-12 and 20 of the specification which describe the process of proteolytic cleavage of α-dystroglycan. Therefore, the need to disclose a genetic mutation in dystroglycan that results in cancer is not seen by Applicants as relevant to the enablement of the invention.

The Examiner also alleges that there is "no guidance as to what level of α-dystroglycan would constitute abnormal levels" in the specification. Applicants respectfully disagree and point to the specification at page 20, which discloses that "the ratios of α- to β-dystroglycan were compared within each sample to measure the amount of α-dystroglycan lost from the cell surface." Support as to the amount of α-dystroglycan shed is also found on page 10, paragraph 1 of the specification, the findings that α-dystroglycan "was found to be greatly reduced or undetectable in the majority" of tumor cell lines tested and that "only those cell lines possessing adequate levels of α-dystroglycan on the cell surface were able to undergo cell rounding."

Further evidence that measurement of α-dystroglycan is indicative of potential tumorigenicity is found on pages 24-25 of the specification, wherein Applicants disclose that restoration of dystroglycan function restores normal cell behavior to tumor cells, and that "cells

possessing restored dystroglycan function did not produce tumors after subcutaneous injection in to the flanks of nude mice", whereas the control cells did.

Applicants respectfully assert that the instant specification provides considerable direction and guidance to a person of relevant skill in the art at the time the application was filed, and also that all of the methods needed to practice the invention are known. Accordingly, Applicants respectfully urge that the rejection be withdrawn.

2. THE CLAIMS ARE NOT OBVIOUS

Claims 22-24 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious in view of Coico *et al.* and Matsumura *et al.* Specifically, that "[s]ince Coico et al. teach the assay method as claimed and Matsumura et al. teach the fragments and antibodies to be used in the method, it would be prima facie obvious to ... combine the teachings to use the method as claimed." (Office Action, page 6, paragraph 13).

There are three basic criteria that must be met to establish a prima facie case of obviousness as laid out in *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991) and MPEP § 2143:

> First, there must be some suggestion or motivation, either in the references themselves or the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest *all* the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure.

In the Office Action, page 6, paragraph 13, the Examiner asserts that "Matsumura et al. teach α-dystroglycan fragments that are approximately 120 and 60 KD." Matsumura et al. (1993) does not teach such fragments. The reference teaches several proteins: dystrophin-associated proteins (DAP) and dystrophin-associated glycoproteins (DAG). 156DAG and 43 DAG correspond to α- and β-dystroglycan respectively. 59DAP, 50DAG, and 35DAG do not

correspond to α-dystroglycan fragments. Upon reading Matsumura et al. (1993), there is no mention of any α-dystroglycan "fragments."

The inventors' novel results demonstrate that α-dystroglycan appears in a 180 kD form on epithelial cell surfaces, and in a 120 kD form in the cell supernatant of the same cell type. The appearance of the smaller form in the culture medium is eliminated by metalloproteinase inhibitors, demonstrating the smaller form results from metalloproteinase activity (not from altered glycosylation) as disclosed on pages 11 and 20-21 of the specification.

Matsumura et al. (1993) also speaks of a 50 kD protein named "50DAG." This protein is not suggested or demonstrated to be a fragment of a-dystroglycan. In fact, a publication also authored by Matsumura identifies this protein as "adhalin." See C R Acad Sci III 1993 Aug; 316(8):799-804). "59DAP" was later disclosed as a molecule called syntrophin. Cell Motil Cytoskeleton 1996;33(3):163-7. The likely identity of "35DAG" is one of the sarcoglycans, as taught in Cell Mol Biol (Noisy-le-grand) 1999 Sep;45(6):751-62.

That these are separate and distinct proteins that were later further characterized in the art is supported by the fact that Matsumura et al. (1993) teaches specific antibodies to each of the above proteins. If these proteins were fragments of α-dystroglycan and not separate proteins then an antibody that recognized 50DAG would have been shown to also recognize 156DAG. Accordingly, the results shown by Matsumura teach that 50DAG, 50DAP, and 35DAG are distinct proteins from α- and β-dystroglycan and not fragments thereof. Therefore, Applicants assert that it would not have been obvious to a person skilled in the relevant art to modify this reference in light of Coico et al. to make the method as claimed in Claims 22-24.

Matsumura et al. (1993) demonstrate that α- and β-dystroglycan, 59DAP and 35 DAG are co-localized with Dp116 and utrophin "in a thin rim surrounding the outermost layer of myelin sheath or peripheral nerve fibers". *Ibid.* at 283. In contrast, Applicants teach that α-dystroglycan is proteolytically cleaved into two fragments and shed into the cell medium. The method of Claims 22-24 requires that the cell medium be assayed for fragments of α-dystroglycan.

Therefore, Matsumura et al. (1993) does not contain all the claim limitations of Applicants' invention.

Matsumura also found that α-dystroglycan has a molecular weight of 120 kD in the peripheral nerve. FEBS, vol. 334, No. 3; 281-285 (1993). The reference teaches that α-dystroglycan appears as a band of 156 kD in skeletal muscle and as 120 kD in sciatic nerve. The two different molecular weights (MWs) forms are not detected in the same cell type. The reason for the difference in molecular weight is presumed to be that "α-dystroglycan might be glycosylated to different levels" in different cell types. Different molecular weights due to variable glycosylation of α-dystroglycan between different cell types has been repeatedly observed by other laboratories. However, no prior art on the record has suggested or demonstrated "fragmentation" of the α-dystroglycan molecule.

Moreover, Mastumura et al. (1993) teaches that the cells should be tested for presence of the proteins, whereas Applicants teach that the cell medium should be tested for the presence of fragments. Matsumura et al. (1993) teaches away from the method of Claims 22-24. Accordingly, the motivation to combine the ELISA method taught by Coico et al. with the teachings of Matsumura et al. (1993) to make Applicants' invention, cannot be found in the references themselves.

Therefore, Applicants respectfully assert that Examiner has not presented a prima facie case for obviousness as laid out in *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). Applicants respectfully request that Examiner withdraw this rejection.

CONCLUSION

Applicants hereby request a One-Month Extension of time from January 2, 2002 to February 2, 2002. A petition for extension of time is included herewith in duplicate. Please charge any necessary and additional fees that may be due to Deposit Account No. 12-0690.

For the reasons set forth above, Applicants respectfully request that a timely Notice of Allowance be issued in this case. Should the Examiner believe that a telephone interview would

aid in the prosecution of this application, Applicants encourage the Examiner to call the undersigned at (510) 495-2839.

Respectfully submitted,

Dated Jan. 3 2002

BY _____

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Appendix 1

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

Please replace the paragraph beginning at page 9, with the following rewritten paragraph. Brackets show omissions; insertions are underlined.

-- Through assays of normal cell function, we have identified dystroglycan as a laminin receptor signaling cytoskeletal and cell shape changes, and cell growth arrest in normal breast epithelial cells. Dystroglycan is a known transmembrane laminin receptor composed of two non-covalently linked portions, α-dystroglycan and β-dystroglycan; see ~~US~~ U.S. Patent Number 5,449,616 hereby incorporated by reference. These originate from a single protein that is post-translationally cleaved. β-dystroglycan is ~~imbedded~~ embedded in the cell membrane. The extracellular chain, α-dystroglycan, binds to laminin. We have shown that inhibition of dystroglycan binding to laminin permits cell spreading and growth in the presence of laminin, conditions where cells would normally round-up and growth arrest. Results suggest a model whereby dystroglycan operates as a co-receptor, which organizes the laminin in the BM and facilitates signaling through other BM receptors. But, dystroglycan is shown to mediate shape changes and growth control without help from β1 and β4 integrins. --

Please replace the paragraph beginning at page 17, with the following rewritten paragraph:

-- Cleavage of α-dystroglycan was detected using cultured cells that cleave and shed dystroglycan from the cell surface. Dystroglycan cleavage was assayed for by ~~immunblotting~~ immunoblotting to detect the presence of dystroglycan fragments in the medium of cultured cells. Mammary carcinoma cell lines SCg6 or TCL1 were cultured in 10 milliliters (ml)

DMEM/F12 medium supplemented with 2% fetal calf serum, 5 μg/ml insulin ~~(Sigma Chemical Co. St. Louis, MO)~~ (Sigma Chemical Co., St. Louis, MO), and 50 μg/ml Gentamycin (UCSF Cell Culture Facility). The cells were allowed to grow to 80% confluence in 10 centimeter (cm) plastic tissue culture dishes. The cells were rinsed two times with phosphate-buffered saline (PBS) and the medium was then changed to 10 mls DMEM/F12 medium supplemented with insulin, and Gentamycin, but without added serum. The cells were incubated in this serum-free medium for 48 ~~hrs~~ hours, then this conditioned medium was harvested and filtered through 0.4 μm filters to remove ~~and~~ cells or cell debris. The harvested medium was concentrated from 10 ml to approximately 1.0 ml using a centriprep-30 concentrator (Amicon, Beverly, MA). The concentrated supernatent was added to 1/10th volume of 10X sample buffer (50% glycerol, 100 mM acetic acid, 10% ~~SDS(w/v)~~ SDS (w/v), 12.5% (v/v) β-mercaptoethanol, bromophenol blue) and heated at 70 degrees Celsius for 15 minutes prior to loading on SDS-polyacrylamide (SDS-PAGE) gels, and subjected to immunoblot analysis. Assays of dystroglycan cleavage and shedding in the presence of the metaloproteinase inhibitor GM6001 (AMS Scientific, Pleasant Hills, CA) were performed in the same manner, with varying concentrations of GM6001, or the control C104 (AMS Scientific), diluted into the serum-free culture medium at the beginning of the 48 hour incubation. To ~~compared~~ compare treatments, equivalent volumes of conditioned medium from each treated cell population were loaded onto the gel to determine the relative quantities of dystroglycan shed into the medium. --

Please replace the last paragraph of the specification that begins on page 24 and carries over to page 25 with the following:

-- The tumorigenic cell line HMT-3522-T4 was found not to round-up in response to laminin when cultured on plastic, indicating that dystroglycan did not function well in these cells. In addition, this cell line is known to not to form organized acinar structures when cultured within a 3-dimensional gel of BM proteins (Matrigel), but instead displays the tumorigenic phenotype of disorganized and uncontrolled cell growth. Therefore, we over-expressed the human dystroglycan gene within these cells to see if, by restoring dystroglycan function, we could

restore normal cell behavior to the tumorigenic T4 cells. Identical cells were also infected with an empty virus control (LXSN). We observed that the cells over-expressing the human dystroglycan gene respond to laminin in the medium by aggregating and rounding, whereas the control cells and rabbit dystroglycan expressing cells respond less. Placing these cells in the 3-D assay show that the T4 cells expressing the human dystroglycan gene no longer display the tumorigenic phenotype, but instead arrest growth and form organized acinar structures. Phase photographs of cultures showed the clear difference in colony size and organization; acinar-like structures were formed by cells over-expressing the dystroglycan cDNA, and disorganized structures are formed by the control population. α6 integrin staining showed the polarization of α6 integrins in dystroglycan over-expressing cells and the lack of polarity in the control population. In addition to reverting the tumorigenic phenotype in culture assays, the cells possessing restored dystroglycan function did not ~~produced~~ produce ~~tumor~~ tumors after subcutaneous injection in to the flanks of nude mice (5×10^6 cells/injection), whereas the control cells did. These results reveal the role of dystroglycan as an important suppressor of tumorigenicity in cells. These results also demonstrate that restoration of dystroglycan to tumor cells is a novel therapeutic approach to slow or reverse the progression of cancer. --